



Novel triazoles of 3-acetylbetulin and betulone as anticancer agents

Ewa Bębenek¹ · Monika Kadela-Tomanek¹ · Elwira Chrobak¹ · Małgorzata Latocha² · Stanisław Boryczka¹

Received: 13 April 2018 / Accepted: 27 June 2018 / Published online: 17 July 2018
© The Author(s) 2018

Abstract

The CuAAC reaction of azides and acetylenic triterpenes was used for synthesis of new triazoles of 3-acetylbetulin and betulone. The triazole derivatives were evaluated for their anticancer activity in vitro against amelanotic melanoma C-32, ductal carcinoma T47D and glioblastoma SNB-19 cell lines. 28-[1-(3'-Deoxythymidine-5'-yl)-1*H*-1,2,3-triazol-4-yl] carbonylbetulone **6e** exhibited a significant IC₅₀ value (0.17 μM) against the human glioblastoma SNB-19 cell line, an almost 5-fold higher potency while compared with reference cisplatin.

Keywords Betulin · 1,2,3-Triazole · CuAAC · Anticancer activity · Lipophilicity

Introduction

The cycloaddition reaction plays an important role in the synthesis of five-membered heterocyclic structures such as 1,2,3-triazoles. Molecules containing a 1,4-disubstituted 1,2,3-triazole ring are prepared regioselectively from azides and terminal alkynes in the copper(I)-catalyzed azide-alkyne cycloaddition reaction CuAAC (Wei et al. 2012; Marciniak et al. 2017). CuAAC reactions, described by Sharpless and Meldal groups, give high yields under mild conditions and have been used to obtain drugs, photo stabilizers and dyes. Additionally, this reaction occurs in various organic solvents and in aqueous media, in a wide pH area. In contrast to the CuAAC reaction, the ruthenium catalyst azide-alkyne cycloaddition is used in the synthesis of the 1,5-disubstituted triazoles (Rostovtsev et al. 2002; Torne et al. 2002; Bonaccorso et al. 2013; Bräse et al. 2008; Totobenazara et al. 2015).

Compounds containing 1,2,3-triazole units exhibit interesting biological activities (antimicrobial, anti-inflammatory, anti-tubercular, and antiviral), which has found numerous applications in bioconjugate chemistry and material science. Additionally, 1,4-disubstituted 1,2,3-triazoles show a significant anticancer activity against human cancer cell lines, which are multidrug-resistant (Wang et al. 2010; Dheer et al. 2017; He et al. 2014).

In the last decades, application of 1,3-dipolar cycloaddition of naturally occurring triterpenes acquired meaning. Conjugation on azides with various alkyne derivatives of pentacyclic triterpenes is designed for the purposes of introduction of the physiologically stable 1,2,3-triazole group (Spivak et al. 2016; Suman et al. 2016; Yu et al. 2013). Most of the triazole analogs of natural compounds have been investigated for their anticancer activity. Majeed et al. synthesized and tested a series of C-3 aryl-substituted 1,2,3-triazoles of betulonic acid for their cytotoxic activity against various human cancer lines like leukemia (HL-60, THP-1), prostate (DU-145, PC-3), lung (A-549), breast (MCF-7), liver (HEP-2), colon (HCT-15), and neuroblastoma (SF-295). The compounds bearing 2-cyanophenyl and 5-hydroxy-1-naphthyl substituted triazole ring exhibited promising IC₅₀ values against HL-60 cell line of 2.5 and 3.5 μM, respectively, in comparison to betulonic acid (IC₅₀ = 17 μM) (Majeed et al. 2013). In the case of C-28 aryl-substituted 1,2,3-triazoles of betulonic acid, it was observed that compounds containing a 4-fluorophenyl substituted triazole ring had the cytotoxic profile similar to that of betulonic acid. This novel triazole hybrid showed a significant antiproliferative activity in HL-60 (leukemia),

✉ Ewa Bębenek
ebebenek@sum.edu.pl

¹ Department of Organic Chemistry, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia in Katowice, 4 Jagiellońska Str., 41-200 Sosnowiec, Poland

² Department of Cell Biology, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia in Katowice, 8 Jedności Str., 41-200 Sosnowiec, Poland

Table 1 The mobile phases used in column chromatography and calculated values of the retention factor

Compound	Mobile phase	Ratio	Retention factor R_f
5a	CH ₂ Cl ₂ –EtOH	60:1	0.39
5b	CH ₂ Cl ₂ –EtOH	40:1	0.55
5c	CH ₂ Cl ₂ –EtOH	60:1	0.32
5d	CH ₂ Cl ₂ –EtOH	60:1	0.43
5e	CHCl ₃ –EtOH	5:1	0.68
5f	CHCl ₃ –EtOH	5:1	0.18
5g	CH ₂ Cl ₂ –EtOH	40:1	0.43
5h	CHCl ₃ –EtOH	15:1	0.45
5i	CHCl ₃ –EtOH	5:1	0.73
6a	CH ₂ Cl ₂ –EtOH	40:1	0.43
6b	CH ₂ Cl ₂ –EtOH	40:1	0.49
6c	CH ₂ Cl ₂ –EtOH	60:1	0.36
6d	CH ₂ Cl ₂ –EtOH	60:1	0.37
6e	CHCl ₃ –EtOH	15:1	0.24
6f	CHCl ₃ –EtOH	5:1	0.22
6g	CH ₂ Cl ₂ –EtOH	60:1	0.31
6h	CHCl ₃ –EtOH	15:1	0.32
6i	CHCl ₃ –EtOH	5:1	0.74
6j	CHCl ₃ –EtOH	5:1	0.20

MIAPACa2 (pancreas), PC-3 (prostate), and A-549 (lung) cell lines, with IC₅₀ values in the range of 5.0–7.0 μM (Khan et al. 2016).

Previously, we described a synthetic route and evaluation of cytotoxicity of betulin and betulone derivatives with a propynoyl group at the C-28 position (Boryczka et al. 2013; Bębenek et al. 2016). Expanding our interest to propynoyl-substituted triterpenes, we converted those acetylenic derivatives into the corresponding 1,2,3-triazoles. In this work, we presented application of the CuAAC reaction in the synthesis of new triazoles of pentacyclic triterpenes and their anticancer activity, as well as lipophilicity properties.

Material and methods

General

All organic solvents (from Sigma-Aldrich and P.P.H. STANLAB) were dried and used after purification. Melting points (m.p.) were determined in open capillary tubes on an Electrothermal IA 9300 melting point apparatus and are uncorrected. The ¹H NMR and ¹³C NMR spectra were recorded on a Bruker Avance III 600 spectrometer in deuterated-d₆ chloroform (CDCl₃) or deuterated-d₆ dimethyl sulfoxide (DMSO) solution. The chemical shifts were reported in ppm (δ), and coupling constant (*J*) values—in hertz (Hz). The spin multiplicity was designated as the

singlet (s), doublet (d), triplet (t), quartet (q), and multiplet (m). High-resolution mass spectra (HR-MS) were recorded on a Bruker Impact II instrument. Infrared spectra (IR) were recorded on a Shimadzu IRAffinity-1 FTIR spectrophotometer (Shimadzu, Japan) using the KBr pellet method. The progress of the reactions was monitored by thin layer chromatography (TLC) using silica gel 60 254 F plates (Merck, Darmstadt, Germany) and detected by spraying with a solution of 5% sulfuric (VI) acid and heating to 120 °C. Purity of the obtained compounds was confirmed by column chromatography carried out on silica gel 60, <63 μm (Merck). A mixture of CHCl₃–EtOH (40:1, 15:1, 5:1 v/v) or CH₂Cl₂–EtOH (60:1, 40:1, v/v) was used as the mobile phase.

Chemistry

Synthesis of 3-acetyl-28-propynoylbetulin 3 and 28-propynoylbetulone 4

3-Acetyl-28-propynoylbetulin **3** was prepared according to the procedure described by Boryczka et al. (Boryczka et al. 2013).

To an ice-cooled (–10 °C) mixture of 3-acetylbetulin **2** (0.48 g, 1 mmol) and propynoic acid (0.12 g, 1.10 mmol) in dichloromethane (5 mL), a freshly prepared solution of dicyclohexylcarbodiimide (0.23 g, 1.12 mmol) and 4-dimethylaminopyridine (0.01 g, 0.08 mmol) in dichloromethane (1 mL) was added. The mixture was allowed to react under argon atmosphere at –10 °C for 5 h. After warming to room temperature, the mixture was stirred overnight. The reaction was monitored by TLC until completion. The resulting precipitate was filtered and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (CHCl₃–EtOH 40:1, v/v).

3-Acetyl-28-propynoylbetulin (3) Yield 79%; mp 115–118 °C; R_f 0.44 (CHCl₃–EtOH, 40:1, v/v); IR (KBr) ν_{\max} 3304, 2946, 2120, 1719, 1457, 1246 cm^{–1}; ¹H NMR (600 MHz, CDCl₃): δ 0.81 (3H, s, CH₃), 0.86 (3H, s, CH₃), 0.87 (3H, s, CH₃), 0.99 (3H, s, CH₃), 1.05 (3H, s, CH₃), 2.07 (3H, s, COCH₃), 2.45 (1H, m, H-19), 2.91 (1H, s, C≡CH), 4.01 (1H, d, *J* = 10.8 Hz, H-28), 4.41 (1H, d, *J* = 10.8 Hz, H-28), 4.48 (1H, m, H-3), 4.62 (1H, s, H-29), 4.71 (1H, s, H-29); ¹³C NMR (150 MHz, CDCl₃): δ 14.7, 16.0, 16.2, 16.5, 18.2, 19.1, 20.8, 21.3, 23.7, 25.1, 27.0, 27.9, 29.5, 29.6, 34.1, 34.4, 37.1, 37.7, 37.8, 38.4, 40.9, 42.7, 46.4, 47.7, 48.8, 50.3, 55.4, 64.9, 74.6, 74.8, 80.9, 110.0, 149.9, 153.3, 171.0; HRAPCIMS *m/z*: 536.3878 C₃₅H₅₂O₄ (calcd. 536.3865).

28-Propynoylbetulone **4** was obtained according to the procedure described by Bębenek et al. The spectra data of

acetylenic ester **4** were consistent with those published in the literature (Bębenek et al. 2016).

General procedure for the synthesis of triazoles **5a–i** and **6a–j**

Based on the previously reported method, the acetylenic esters **3–4** were converted into triazoles **5a–i** and **6a–j** by reactions with organic azides in toluene in the presence of copper(I) iodide (Bębenek et al. 2017). The copper(I) iodide (0.1 eqv, 0.004 g, 0.02 mmol) and the organic azide (1.05 eqv, 0.21 mmol) were added to a solution of propynoylated derivatives **3** or **4** (0.20 mmol) in toluene (4 mL). Next, the reaction mixture was stirred for another 72 h under reflux. The solvent was evaporated. The crude residue was purified by silica gel column chromatography using various mixtures of organic solvents. The same mobile phases were applied for TLC and in column chromatography (Table 1).

3-Acetyl-28-(1-benzyl-1H-1,2,3-triazol-4-yl)carbonylbetulin (5a) Yield 73%; m.p. 109–111 °C; IR (KBr) ν_{\max} 3134, 2947, 1732, 1527, 1456, 1246–1193 cm^{-1} ; ^1H NMR (600 MHz, CDCl_3): δ 0.85 (3H, s, CH_3), 0.86 (3H, s, CH_3), 0.87 (3H, s, CH_3), 0.99 (3H, s, CH_3), 1.06 (3H, s, CH_3), 1.71 (3H, s, CH_3), 2.06 (3H, s, COCH_3), 2.51 (1H, m, H-19), 4.13 (1H, d, $J = 10.8$ Hz, H-28), 4.49 (1H, m, H-3), 4.55 (1H, d, $J = 10.8$ Hz, H-28), 4.62 (1H, s, H-29), 4.72 (1H, s, H-29), 5.60 (2H, s, CH_2), 7.31–7.33 (2H, m, H_{Ar}), 7.41–7.44 (3H, m, H_{Ar}), 7.97 (1H, s, CH-triazole); ^{13}C NMR (150 MHz, CDCl_3): δ 14.2, 14.7, 16.0, 16.2, 16.5, 18.2, 19.1, 20.8, 21.1, 21.3, 23.7, 25.2, 27.1, 27.9, 29.6, 29.8, 34.1, 34.7, 37.1, 37.7, 38.4, 40.9, 42.7, 46.7, 47.7, 48.9, 50.3, 54.5, 55.4, 60.4, 63.6, 80.9, 109.9, 127.1, 128.2, 129.2, 129.3, 133.8, 140.6, 150.1, 161.2, 171.1; HRAP-CIMS m/z (neg): 668.4474 $\text{C}_{42}\text{H}_{58}\text{N}_3\text{O}_4$ (calcd. 668.4427).

3-Acetyl-28-[1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl]carbonylbetulin (5b) Yield 63%; m.p. 113–116 °C; IR (KBr) ν_{\max} 3138, 2963, 1734, 1539, 1457, 1226–1193, 802 cm^{-1} ; ^1H NMR (600 MHz, CDCl_3): δ 0.85 (3H, s, CH_3), 0.86 (3H, s, CH_3), 0.87 (3H, s, CH_3), 1.00 (3H, s, CH_3), 1.06 (3H, s, CH_3), 1.69 (3H, s, CH_3), 2.07 (3H, s, COCH_3), 2.52 (1H, m, H-19), 4.14 (1H, d, $J = 10.8$ Hz, H-28), 4.49 (1H, m, H-3), 4.57 (1H, d, $J = 10.8$ Hz, H-28), 4.62 (1H, s, H-29), 4.72 (1H, s, H-29), 5.57 (2H, s, CH_2), 7.10–7.13 (2H, m, H_{Ar}), 7.31–7.33 (2H, m, H_{Ar}), 7.97 (1H, s, CH-triazole); ^{13}C NMR (150 MHz, CDCl_3): δ 13.7, 15.1, 15.2, 15.5, 17.1, 18.1, 19.8, 20.3, 22.7, 24.1, 26.1, 26.9, 28.6, 28.8, 33.1, 33.7, 36.0, 36.6, 36.8, 37.4, 39.9, 41.7, 45.6, 46.7, 47.8, 49.3, 52.7, 54.4, 62.6, 79.9, 108.9, 115.3, 115.4, 125.9, 129.1, 129.2, 139.7, 149.0, 160.0, 161.2, 170.0; HRAP-CIMS m/z (neg): 686.4357 $\text{C}_{42}\text{H}_{57}\text{FN}_3\text{O}_4$ (calcd. 686.4333).

3-Acetyl-28-[1-(4-cyanobenzyl)-1H-1,2,3-triazol-4-yl]carbonylbetulin (5c) Yield 56%; m.p. 137–140 °C; IR (KBr) ν_{\max} 3144, 2949, 2231, 1734, 1540, 1457, 1248–1192 cm^{-1} ; ^1H NMR (600 MHz, CDCl_3): δ 0.83 (3H, s, CH_3), 0.86 (3H, s, CH_3), 0.87 (3H, s, CH_3), 1.00 (3H, s, CH_3), 1.07 (3H, s, CH_3), 1.70 (3H, s, CH_3), 2.07 (3H, s, COCH_3), 2.52 (1H, m, H-19), 4.16 (1H, d, $J = 10.8$ Hz, H-28), 4.49 (1H, m, H-3), 4.58 (1H, d, $J = 10.8$ Hz, H-28), 4.63 (1H, s, H-29), 4.72 (1H, s, H-29), 5.68 (2H, s, CH_2), 7.40 (2H, d, $J = 8.4$ Hz, H_{Ar}), 7.72 (2H, d, $J = 8.4$ Hz, H_{Ar}), 8.04 (1H, s, CH-triazole); ^{13}C NMR (150 MHz, CDCl_3): δ 14.8, 16.0, 16.2, 16.5, 18.2, 19.1, 19.3, 20.8, 21.3, 23.7, 25.2, 27.1, 27.9, 29.6, 29.8, 34.1, 34.7, 37.1, 37.7, 37.8, 38.4, 40.9, 42.7, 46.7, 47.7, 48.9, 50.3, 53.7, 55.4, 63.8, 80.9, 110.3, 113.3, 117.9, 124.4, 127.3, 128.5, 133.1, 138.9, 141.0, 150.0, 160.9, 171.0; HRAP-CIMS m/z (neg): 693.4352 $\text{C}_{43}\text{H}_{57}\text{N}_4\text{O}_4$ (calcd. 693.4380).

3-Acetyl-28-(1-phenylthiomethyl-1H-1,2,3-triazol-4-yl)carbonylbetulin (5d) Yield 60%; m.p. 105–107 °C; IR (KBr) ν_{\max} 2945, 1734, 1539, 1456, 1247–1194 cm^{-1} ; ^1H NMR (600 MHz, CDCl_3): δ 0.86 (3H, s, CH_3), 0.87 (3H, s, CH_3), 0.88 (3H, s, CH_3), 1.01 (3H, s, CH_3), 1.09 (3H, s, CH_3), 1.71 (3H, s, CH_3), 2.07 (3H, s, COCH_3), 2.51 (1H, m, H-19), 4.14 (1H, d, $J = 10.8$ Hz, H-28), 4.49 (1H, m, H-3), 4.55 (1H, d, $J = 10.8$ Hz, H-28), 4.63 (1H, s, H-29), 4.73 (1H, s, H-29), 5.68 (2H, s, CH_2), 7.35–7.36 (5H, m, H_{Ar}), 8.04 (1H, s, CH-triazole); ^{13}C NMR (150 MHz, CDCl_3): δ 14.8, 16.1, 16.2, 16.5, 18.2, 19.1, 20.8, 21.3, 23.7, 25.2, 25.6, 27.1, 27.9, 29.6, 29.8, 34.1, 34.7, 37.1, 37.7, 37.8, 38.4, 40.9, 42.7, 46.7, 47.8, 48.9, 50.3, 54.3, 55.4, 63.6, 68.0, 80.9, 110.0, 126.8, 129.1, 129.7, 131.3, 132.4, 140.6, 150.0, 160.9, 171.0; HRAP-CIMS m/z (neg): 700.4141 $\text{C}_{42}\text{H}_{58}\text{N}_3\text{O}_4\text{S}$ (calcd. 700.4148).

3-Acetyl-28-[1-(3'-deoxythymidine-5'-yl)-1H-1,2,3-triazol-4-yl]carbonylbetulin (5e) Yield 65%; m.p. 204–207 °C; IR (KBr) ν_{\max} 3446, 3068, 2945, 1730, 1541, 1456, 1246–1192 cm^{-1} ; ^1H NMR (600 MHz, $\text{DMSO}-d_6$): δ 0.81 (3H, s, CH_3), 0.83 (3H, s, CH_3), 0.98 (3H, s, CH_3), 1.02 (3H, s, CH_3), 1.18 (3H, s, CH_3), 1.67 (3H, s, CH_3), 1.78 (3H, s, CH_3 -AZT), 2.03 (3H, s, COCH_3), 2.55 (1H, m, AZT), 2.67 (1H, m, H-19), 3.65–3.70 (2H, m, AZT), 4.03 (1H, d, $J = 10.8$ Hz, H-28), 4.27 (1H, t, $J = 4.8$ Hz, AZT), 4.38 (1H, m, H-3), 4.55 (1H, d, $J = 10.8$ Hz, H-28), 4.59 (1H, s, H-29), 4.73 (1H, s, H-29), 5.28 (1H, t, $J = 4.8$ Hz, AZT), 5.46 (1H, m, AZT), 6.44 (1H, t, $J = 6.6$ Hz, AZT), 7.82 (1H, s, AZT), 8.32 (1H, s, CH-triazole), 9.01 (1H, s, NH-AZT); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$): δ 12.7, 14.9, 15.9, 16.1, 16.3, 16.9, 18.2, 19.3, 20.8, 21.5, 23.8, 25.3, 27.1, 28.1, 29.4, 29.7, 34.0, 34.6, 37.1, 37.6, 37.7, 37.8, 38.2, 42.8, 46.8, 47.5, 48.7, 49.9, 55.0, 55.6, 60.2, 61.1, 62.7, 79.6, 80.4, 84.6, 110.1, 110.5, 129.3, 136.7, 139.3,

150.9, 160.9, 164.2; 170.6; HRAPCIMS m/z (neg): 802.4768 C₄₅H₆₄N₅O₈ (calcd. 802.4755).

3-Acetyl-28-[1-(1-deoxy- β -D-glucopyranosyl)-1H-1,2,3-triazol-4-yl]carbonylbetulin (5f) Yield 82%; m.p. 210–212 °C; IR (KBr) ν_{\max} 3419, 2943, 1732, 1543, 1456, 1246–1191 cm⁻¹; ¹H NMR (600 MHz, DMSO-d₆): δ 0.80 (3H, s, CH₃), 0.81 (3H, s, CH₃), 0.83 (3H, s, CH₃), 0.98 (3H, s, CH₃), 1.04 (3H, s, CH₃), 1.68 (3H, s, CH₃), 1.85 (1H, m, OH), 2.00 (3H, s, COCH₃), 2.54 (1H, m, H-19), 3.27 (1H, m, OH), 3.39 (1H, m, OH), 3.44 (1H, m, OH), 3.71 (1H, m, CH-sugar), 3.85 (1H, m, CH-sugar), 4.03 (1H, d, J = 10.8 Hz, H-28), 4.37 (1H, m, H-3), 4.55 (1H, d, J = 10.8 Hz, H-28), 4.59 (1H, s, H-29), 4.63 (1H, m, CH-sugar), 4.73 (1H, s, H-29), 5.20 (1H, d, J = 5.4 Hz, CH-sugar), 5.35 (1H, d, J = 5.4 Hz, CH-sugar), 5.46 (1H, d, J = 5.4 Hz, CH-sugar), 5.61 (1H, d, J = 5.4 Hz, CH-sugar), 9.08 (1H, s, CH-triazole); ¹³C NMR (150 MHz, DMSO-d₆): δ 15.0, 16.1, 16.3, 16.9, 18.2, 19.3, 20.8, 21.5, 23.8, 25.2, 27.1, 28.1, 29.4, 29.6, 34.0, 34.6, 37.1, 37.6, 37.8, 38.2, 39.6, 42.8, 46.8, 47.5, 48.7, 50.0, 55.0, 61.2, 62.6, 69.9, 72.4, 77.2, 79.6, 80.6, 88.3, 110.5, 129.1, 139.2, 150.3, 160.9, 170.6; HRAPCIMS m/z (neg): 740.4480 C₄₁H₆₂N₃O₉ (calcd. 740.4486).

3-Acetyl-28-(1-ethylacetyl-1H-1,2,3-triazol-4-yl)carbonylbetulin (5g) Yield 80%; m.p. 221–224 °C; IR (KBr) ν_{\max} 2945, 1732, 1544, 1465, 1247–1213 cm⁻¹; ¹H NMR (600 MHz, CDCl₃): δ 0.85 (3H, s, CH₃), 0.86 (3H, s, CH₃), 0.88 (3H, s, CH₃), 0.99 (3H, s, CH₃), 1.08 (3H, s, CH₃), 1.34 (3H, t, J = 7.2 Hz, CH₃), 1.68 (3H, s, CH₃), 2.07 (3H, s, COCH₃), 2.53 (1H, m, H-19), 4.18 (1H, d, J = 10.8 Hz, H-28), 4.32 (2H, q, J = 7.2 Hz, OCH₂), 4.48 (1H, m, H-3), 4.58 (1H, d, J = 10.8 Hz, H-28), 4.63 (1H, s, H-29), 4.73 (1H, s, H-29), 5.24 (2H, s, CH₂), 8.24 (1H, s, CH-triazole); ¹³C NMR (150 MHz, CDCl₃): δ 14.1, 14.8, 16.1, 16.2, 16.5, 18.2, 20.8, 21.3, 23.7, 25.2, 27.1, 27.9, 29.6, 29.8, 34.1, 34.7, 37.1, 37.7, 37.8, 38.4, 40.9, 42.7, 46.7, 47.8, 48.9, 50.3, 51.0, 55.4, 62.8, 63.7, 80.9, 110.0, 128.7, 140.7, 150.1, 160.9, 165.7, 171.1; HRAPCIMS m/z (neg): 664.4329 C₃₉H₅₈N₃O₆ (calcd. 664.4326).

3-Acetyl-28-[1-(3-hydroxypropyl)-1H-1,2,3-triazol-4-yl]carbonylbetulin (5h) Yield 83%; m.p. 116–119 °C; IR (KBr) ν_{\max} 3425, 2945, 1732, 1543, 1465, 1246–1199 cm⁻¹; ¹H NMR (600 MHz, DMSO-d₆): δ 0.80 (3H, s, CH₃), 0.81 (3H, s, CH₃), 0.82 (3H, s, CH₃), 0.98 (3H, s, CH₃), 1.03 (3H, s, CH₃), 1.09 (2H, m, CH₂), 1.67 (3H, s, CH₃), 2.02 (3H, s, COCH₃), 2.51 (1H, m, H-19), 3.34 (2H, m, CH₂), 4.01 (1H, d, J = 10.8 Hz, H-28), 4.38 (1H, m, H-3), 4.48 (2H, t, J = 7.2 Hz, CH₂), 4.54 (1H, d, J = 10.8 Hz, H-28), 4.59 (1H, s, H-29), 4.73 (1H, s, H-29), 8.81 (1H, s, CH-triazole); ¹³C NMR (150 MHz, DMSO-d₆): δ 14.9, 16.1, 16.3, 16.9, 18.2,

19.2, 20.7, 21.5, 24.8, 25.1, 27.1, 28.1, 29.4, 29.6, 33.1, 34.0, 34.6, 37.1, 37.6, 37.8, 38.2, 40.9, 42.8, 46.8, 47.5, 47.6, 48.7, 50.0, 55.0, 57.8, 62.5, 80.4, 110.4, 129.7, 139.0, 150.3, 161.0, 170.6; HRAPCIMS m/z (neg): 636.4363 C₃₈H₅₈N₃O₅ (calcd. 636.4376).

2-Amino-3-[4-(3-acetyl-28-betulinylcarbonyl)-1H-1,2,3-triazol-1-yl]propanoic acid (5i) Yield 45%; oil; IR (KBr) ν_{\max} 3444, 2956, 1732, 1602, 1458, 1246–1122 cm⁻¹; ¹H NMR (600 MHz, DMSO-d₆): δ : 0.80 (3H, s, CH₃), 0.82 (3H, s, CH₃), 0.86 (3H, s, CH₃), 0.92 (3H, s, CH₃), 1.01 (3H, s, CH₃), 1.06 (1H, t, J = 7.2 Hz, CH), 1.67 (3H, s, CH₃), 2.00 (3H, s, COCH₃), 2.52 (1H, m, H-19), 4.14 (1H, d, J = 10.8 Hz, H-28), 4.23 (2H, d, J = 7.2 Hz, CH₂), 4.37 (1H, m, H-3), 4.55 (1H, d, J = 10.8 Hz, H-28), 4.58 (1H, s, H-29), 4.73 (1H, s, H-29), 8.62 (1H, s, CH-triazole); ¹³C NMR (150 MHz, DMSO-d₆): δ 14.9, 15.8, 16.1, 16.8, 18.3, 19.2, 19.6, 21.2, 22.9, 23.7, 26.8, 28.8, 30.3, 30.6, 30.8, 34.1, 34.5, 36.8, 37.2, 37.5, 38.5, 39.6, 42.8, 46.2, 47.0, 47.2, 48.6, 50.1, 54.3, 56.5, 67.8, 80.2, 110.0, 129.2, 132.1, 150.1, 161.0, 167.5; HRAPCIMS m/z (neg): 665.4269 C₃₈H₅₇N₄O₆ (calcd. 665.4278).

28-(1-Benzyl-1H-1,2,3-triazol-4-yl)carbonylbetulone (6a) Yield 81%; m.p. 196–198 °C; IR (KBr) ν_{\max} 2963, 1738, 1700, 1539, 1465, 1261–1193 cm⁻¹; ¹H NMR (600 MHz, CDCl₃): δ : 0.94 (3H, s, CH₃), 1.02 (3H, s, CH₃), 1.05 (3H, s, CH₃), 1.10 (3H, s, CH₃), 1.12 (3H, s, CH₃), 1.69 (3H, s, CH₃), 2.52 (1H, m, H-19), 4.15 (1H, d, J = 10.8 Hz, H-28), 4.57 (1H, d, J = 10.8 Hz, H-28), 4.62 (1H, s, H-29), 4.72 (1H, s, H-29), 5.60 (2H, s, CH₂), 7.31–7.33 (2H, m, H_{Ar}), 7.41–7.44 (3H, m, H_{Ar}), 7.97 (1H, s, CH-triazole); ¹³C NMR (150 MHz, CDCl₃): δ 14.7, 15.8, 15.9, 19.1, 19.6, 21.1, 21.3, 25.2, 25.6, 26.6, 27.1, 29.6, 29.8, 33.5, 34.2, 34.7, 36.9, 37.8, 39.6, 40.9, 42.8, 46.7, 47.4, 47.7, 48.8, 49.7, 54.5, 55.0, 63.5, 68.0, 110.0, 127.1, 128.2, 129.2, 129.3, 133.8, 140.6, 150.0, 161.2, 218.0; HRAPCIMS m/z (neg): 624.4171 C₄₀H₅₄N₃O₃ (calcd. 624.4165).

28-[1-(4-Fluorobenzyl)-1H-1,2,3-triazol-4-yl]carbonylbetulone (6b) Yield 73%; m.p. 220–223 °C; IR (KBr) ν_{\max} 3131, 2957, 1742, 1699, 1539, 1456, 1223–1198, 814 cm⁻¹; ¹H NMR (600 MHz, CDCl₃): δ 0.86 (3H, s, CH₃), 0.91 (3H, s, CH₃), 0.92 (3H, s, CH₃), 0.95 (3H, s, CH₃), 1.02 (3H, s, CH₃), 1.68 (3H, s, CH₃), 2.43 (1H, m, H-19), 4.05 (1H, d, J = 10.8 Hz, H-28), 4.48 (1H, d, J = 10.8 Hz, H-28), 4.53 (1H, s, H-29), 4.63 (1H, s, H-29), 5.48 (2H, s, CH₂), 7.01–7.04 (2H, m, H_{Ar}), 7.23–7.36 (2H, m, H_{Ar}), 7.88 (1H, s, CH-triazole); ¹³C NMR (150 MHz, CDCl₃): δ 14.7, 15.8, 15.9, 19.2, 19.6, 21.1, 21.3, 25.2, 26.6, 27.1, 29.6, 29.8, 33.5, 34.2, 34.7, 36.9, 37.8, 39.6, 40.9, 42.8, 46.7, 47.4, 47.7, 48.8, 49.7, 53.7, 55.0, 63.6, 68.1, 110.0, 116.3, 116.5,

126.2, 126.3, 130.2, 140.0, 150.0, 161.1, 218.0; HRAPCIMS m/z (neg): 642.4063 $C_{40}H_{53}FN_3O_3$ (calcd. 642.4071).

28-[1-(4-Cyanobenzyl)-1H-1,2,3-triazol-4-yl]carbonylbetulone (6c) Yield 57%; m.p. 211–214 °C; IR (KBr) ν_{\max} 3127, 2951, 2229, 1734, 1705, 1525, 1457, 1243–1147 cm^{-1} ; 1H NMR (600 MHz, $CDCl_3$): δ 0.87 (3H, s, CH_3), 0.93 (3H, s, CH_3), 0.96 (3H, s, CH_3), 1.02 (3H, s, CH_3), 1.03 (3H, s, CH_3), 1.68 (3H, s, CH_3), 2.44 (1H, m, H-19), 4.07 (1H, d, $J = 10.8$ Hz, H-28), 4.49 (1H, d, $J = 10.8$ Hz, 1H, H-28), 4.54 (1H, s, H-29), 4.63 (1H, s, H-29), 5.59 (2H, s, CH_2), 7.31 (2H, d, $J = 8.4$ Hz, H_{Ar}), 7.63 (2H, d, $J = 8.4$ Hz, H_{Ar}), 7.96 (1H, s, CH-triazole); ^{13}C NMR (150 MHz, $CDCl_3$): δ 14.7, 15.8, 15.9, 19.1, 19.6, 21.1, 21.3, 23.7, 25.2, 26.6, 27.1, 29.6, 29.8, 33.5, 34.2, 34.7, 36.9, 37.8, 39.6, 40.9, 42.8, 46.7, 47.4, 47.7, 48.8, 49.7, 53.7, 55.0, 63.7, 110.1, 113.3, 117.9, 127.4, 128.5, 133.1, 138.9, 141.0, 149.9, 160.9, 218.1; HRAPCIMS m/z (neg): 649.4095 $C_{41}H_{53}N_4O_3$ (calcd. 649.4118).

28-(1-Phenylthiomethyl-1H-1,2,3-triazol-4-yl)carbonylbetulone (6d) Yield 87%; m.p. 188–191 °C; IR (KBr) ν_{\max} 3132, 2960, 1734, 1705, 1521, 1456, 1241–1196 cm^{-1} ; 1H NMR (600 MHz, $CDCl_3$): δ 0.96 (3H, s, CH_3), 1.03 (3H, s, CH_3), 1.05 (3H, s, CH_3), 1.09 (3H, s, CH_3), 1.11 (3H, s, CH_3), 1.69 (3H, s, CH_3), 2.53 (1H, m, H-19), 4.15 (1H, d, $J = 10.8$ Hz, H-28), 4.57 (1H, d, $J = 10.8$ Hz, H-28), 4.63 (1H, s, H-29), 4.74 (1H, s, H-29), 5.69 (2H, s, CH_2), 7.34–7.37 (5H, m, H_{Ar}), 8.06 (1H, s, CH-triazole); ^{13}C NMR (150 MHz, $CDCl_3$): δ 14.7, 15.8, 15.9, 19.1, 19.6, 21.1, 21.3, 25.2, 25.6, 26.6, 27.1, 29.6, 29.8, 33.5, 34.2, 34.7, 36.9, 37.8, 39.6, 40.9, 42.8, 46.7, 47.4, 47.7, 48.8, 49.7, 54.3, 55.0, 63.6, 68.0, 110.0, 126.8, 129.1, 129.7, 131.3, 132.4, 150.0, 160.9, 218.1; HRAPCIMS m/z (neg): 656.3895 $C_{40}H_{54}N_3O_3S$ (calcd. 656.3886).

28-[1-(3'-Deoxythymidine-5'-yl)-1H-1,2,3-triazol-4-yl]carbonylbetulone (6e) Yield 73%; m.p. 199–202 °C; IR (KBr) ν_{\max} 3447, 3068, 2945, 1729, 1697, 1541, 1458, 1226–1163 cm^{-1} ; 1H NMR (600 MHz, $DMSO-d_6$): δ 0.88 (3H, s, CH_3), 0.94 (3H, s, CH_3), 0.99 (3H, s, CH_3), 1.02 (3H, s, CH_3), 1.05 (3H, s, CH_3), 1.68 (3H, s, CH_3), 1.74 (3H, s, CH_3 -AZT), 2.51 (1H, m, AZT), 2.67 (1H, m, H-19), 3.65–3.70 (2H, m, AZT), 4.04 (1H, d, $J = 10.8$ Hz, H-28), 4.27 (1H, t, $J = 4.8$ Hz, AZT), 4.55 (1H, d, $J = 10.8$ Hz, H-28), 4.58 (1H, s, H-29), 4.74 (1H, s, H-29), 5.27 (1H, t, $J = 4.8$ Hz, AZT), 5.46 (1H, m, AZT), 6.44 (1H, t, $J = 6.6$ Hz, AZT), 7.82 (1H, s, AZT), 8.32 (1H, s, CH-triazole), 9.02 (1H, s, NH-AZT); ^{13}C NMR (150 MHz, $DMSO-d_6$): δ 12.7, 14.9, 15.8, 15.9, 16.1, 19.3, 19.6, 21.2, 21.3, 25.3, 26.8, 27.1, 29.4, 29.6, 33.4, 34.1, 34.6, 36.8, 37.6, 37.7, 39.3, 39.6, 42.8, 46.8, 47.0, 47.5, 48.6, 49.4, 54.3, 55.6, 60.2, 61.1, 62.7, 79.6, 84.3, 84.7, 110.1, 110.5, 129.3, 136.7,

139.3, 150.3, 150.9, 160.9, 164.2, 217.0; HRAPCIMS m/z (neg): 758.4484 $C_{43}H_{60}N_5O_7$ (calcd. 758.4493).

28-[1-(1-Deoxy- β -D-glucopyranosyl)-1H-1,2,3-triazol-4-yl]carbonylbetulone (6f) Yield 74%; m.p. 187–189 °C; IR (KBr) ν_{\max} 3419, 2939, 1732, 1701, 1541, 1458, 1232–1190 cm^{-1} ; 1H NMR (600 MHz, $DMSO-d_6$): δ 0.88 (3H, s, CH_3), 0.94 (3H, s, CH_3), 0.99 (3H, s, CH_3), 1.02 (3H, s, CH_3), 1.07 (3H, s, CH_3), 1.68 (3H, s, CH_3), 1.86 (1H, m, OH), 2.56 (1H, m, H-19), 3.27 (1H, m, OH), 3.40 (1H, m, OH), 3.45 (1H, m, OH), 3.71 (1H, m, CH-sugar), 3.85 (1H, m, CH-sugar), 4.04 (1H, d, $J = 10.8$ Hz, H-28), 4.56 (1H, d, $J = 10.8$ Hz, H-28), 4.59 (1H, s, H-29), 4.63 (1H, m, CH-sugar), 4.74 (1H, s, H-29), 5.20 (1H, d, $J = 5.4$ Hz, CH-sugar), 5.34 (1H, d, $J = 5.4$ Hz, CH-sugar), 5.45 (1H, d, $J = 5.4$ Hz, CH-sugar), 5.62 (1H, d, $J = 5.4$ Hz, CH-sugar), 9.08 (1H, s, CH-triazole); ^{13}C NMR (150 MHz, $DMSO-d_6$): δ 14.9, 15.8, 16.1, 19.3, 19.6, 21.2, 21.3, 25.2, 26.8, 27.1, 29.4, 29.6, 33.4, 34.1, 34.6, 36.8, 37.1, 39.3, 42.8, 46.8, 47.0, 47.5, 48.7, 49.4, 54.3, 61.2, 62.6, 69.9, 72.4, 77.2, 79.6, 80.6, 88.3, 110.5, 129.1, 139.2, 150.3, 160.9, 218.0; HRAPCIMS m/z (neg): 696.4220 $C_{39}H_{58}N_3O_8$ (calcd. 696.4224).

28-(1-Ethylacetyl-1H-1,2,3-triazol-4-yl)carbonylbetulone (6g) Yield 79%; m.p. 97–99 °C; IR (KBr) ν_{\max} 3147, 2945, 1755, 1705, 1541, 1458, 1211–1111 cm^{-1} ; 1H NMR (600 MHz, $CDCl_3$): δ 0.88 (3H, s, CH_3), 0.93 (3H, s, CH_3), 0.95 (3H, s, CH_3), 1.00 (3H, s, CH_3), 1.03 (3H, s, CH_3), 1.26 (3H, t, $J = 7.2$ Hz, CH_3), 1.68 (3H, s, CH_3), 2.45 (1H, m, H-19), 4.10 (1H, d, $J = 10.8$ Hz, H-28), 4.23 (2H, q, $J = 7.2$ Hz, OCH_2), 4.51 (1H, d, $J = 10.8$ Hz, H-28), 4.54 (1H, s, H-29), 4.65 (1H, s, H-29), 5.15 (2H, s, CH_2), 8.16 (1H, s, CH-triazole); ^{13}C NMR (150 MHz, $CDCl_3$): δ 14.2, 14.7, 15.9, 19.6, 21.1, 21.3, 25.2, 26.6, 27.1, 29.6, 29.8, 33.5, 34.2, 34.7, 36.9, 37.8, 39.6, 40.9, 42.8, 46.7, 47.4, 47.7, 48.8, 49.7, 51.0, 55.0, 60.4, 62.8, 63.6, 110.0, 128.7, 140.7, 150.1, 160.9, 165.7, 171.2, 218.1; HRAPCIMS m/z (neg): 620.4049 $C_{37}H_{54}N_3O_5$ (calcd. 620.4063).

28-[1-(3-Hydroxypropyl)-1H-1,2,3-triazol-4-yl]carbonylbetulone (6h) Yield 78%; m.p. 197–199 °C; IR (KBr) ν_{\max} 3404, 2960, 1735, 1703, 1543, 1458, 1261–1223 cm^{-1} ; 1H NMR (600 MHz, $DMSO-d_6$): δ 0.88 (3H, s, CH_3), 0.94 (3H, s, CH_3), 0.99 (3H, s, CH_3), 1.00 (3H, s, CH_3), 1.05 (3H, s, CH_3), 1.10 (2H, m, CH_2), 1.67 (3H, s, CH_3), 2.52 (1H, m, H-19), 3.38 (2H, m, CH_2), 4.02 (1H, d, $J = 10.8$ Hz, H-28), 4.48 (2H, t, $J = 7.2$ Hz, CH_2), 4.55 (1H, d, $J = 10.8$ Hz, H-28), 4.59 (1H, s, H-29), 4.74 (1H, s, H-29), 8.81 (1H, s, CH-triazole); ^{13}C NMR (150 MHz, $DMSO-d_6$): δ 14.9, 15.8, 16.1, 19.2, 19.5, 21.2, 21.3, 25.2, 26.8, 27.1, 29.4, 29.6, 33.1, 33.4, 34.1, 34.6, 36.8, 37.7, 39.3, 42.8, 46.8, 47.0, 47.5, 47.6, 48.6, 49.3, 54.3, 57.8, 62.5, 79.6, 110.5, 129.7,

139.0, 150.3 161.0, 217.0; HRAPCIMS m/z (neg): 592.4131 C₃₆H₅₄N₃O₄ (calcd. 592.4114).

2-Amino-3-[4-(3-acetyl-28-betulononylcarbonyl)-1H-1,2,3-triazol-1-yl]propanoic acid (6i) Yield 48%; 163–166 °C; IR (KBr) ν_{\max} 3479, 2956, 1732, 1705, 1606, 1456, 1280–1223 cm⁻¹; ¹H NMR (600 MHz, DMSO-d₆) δ : 0.85 (3 H s, CH₃), 0.89 (3H, s, CH₃), 0.98 (3H, s, CH₃), 1.02 (3H, s, CH₃), 1.04 (3H, s, CH₃), 1.07 (1H, t, $J = 7.2$ Hz, CH), 1.67 (3H, s, CH₃), 2.51 (1H, m, H-19), 4.12 (1H, d, $J = 10.8$ Hz, H-28), 4.21 (2H, d, $J = 7.2$ Hz, CH₂), 4.56 (1H, d, $J = 10.8$ Hz, H-28), 4.59 (1H, s, H-29), 4.74 (1H, s, H-29), 8.62 (1H, s, CH-triazole); ¹³C NMR (150 MHz, DMSO-d₆): δ 14.3, 15.0, 16.1, 16.3, 16.9, 21.5, 22.9, 23.7, 28.1, 28.8, 30.2, 37.1, 37.8, 38.5, 39.5, 40.8, 42.8, 67.9, 80.4, 110.0, 129.1, 132.1, 132.2, 150.1, 167.5, 217.1; HRAPCIMS m/z (neg): 621.4050 C₃₆H₅₃N₄O₅ (calcd. 621.4015).

3-Methyl-3-[4-(28-betulononylcarbonyl)-1H-1,2,3-triazol-1-yl]butyric acid (6j) Yield 54%; m.p. 246–249 °C; IR (KBr) ν_{\max} 3446, 2945, 1732, 1709, 1616, 1456, 1280–1211 cm⁻¹; ¹H NMR (600 MHz, DMSO-d₆) δ : 0.72 (3H, s, CH₃), 0.87 (3H, s, CH₃), 0.96 (3H, s, CH₃), 1.00 (3H, d, $J = 6.6$ Hz, CH₃), 1.01 (3H, s, CH₃), 1.04 (3H, s, CH₃), 1.08 (3H, d, $J = 6.6$ Hz, CH₃), 1.68 (3H, s, CH₃), 2.43 (1H, m, CH), 2.53 (1H, m, H-19), 4.03 (1H, d, $J = 10.8$ Hz, H-28), 4.28 (1H, m, CHCOOH), 4.55 (1H, d, $J = 10.8$ Hz, H-28), 4.64 (1H, s, H-29), 4.74 (1H, s, H-29), 8.76 (1H, s, CH-triazole); ¹³C NMR (150 MHz, DMSO-d₆) δ : 14.3, 14.6, 14.9, 15.8, 16.0, 16.1, 18.9, 19.2, 19.6, 19.9, 21.2, 25.2, 26.8, 27.1, 29.5, 29.7, 30.8, 33.4, 34.1, 36.8, 37.7, 39.3, 40.8, 42.8, 46.8, 47.0, 47.5, 48.6, 49.3, 54.3, 62.6, 79.7, 110.5, 129.4, 138.7, 150.3, 161.3, 217.1; HRAPCIMS m/z (neg): 535.3878 C₃₅H₅₁O₄ (calcd. 535.3865).

Biological study

Cells

The triterpenes were evaluated for their cytotoxic activity towards three human cancer cell lines: amelanotic melanoma C-32 (ATCC, Rockville, USA), ductal carcinoma T47D (ATCC, Rockville, USA) and glioblastoma SNB-19 (DSMZ, Braunschweig, Germany). The cells were seeded in 96-well plates (Nunc Thermo Fisher Scientific, Waltham, USA) at a density of 5×10^3 cells per well and maintained for 24 h at 37 °C in a humid atmosphere saturated with 5% CO₂. All cancer cell lines were cultured in DMEM (Lonza, Basel, Switzerland) growth medium containing 10% fetal bovine serum (FBS) (Biological Industries, Cromwell, USA), penicillin (10,000 U/mL) and streptomycin (10 mg/mL) (Lonza, Basel, Switzerland).

WST-1 assay

A WST-1 assay (Roche Diagnostics GmbH, Mannheim, Germany) was used for the evaluate of cytotoxicity against the tested human cancer cell lines. The WST-1 assay was carried out after 72 h incubation of the cells with concentrations ranging from 1 to 100 μ g/mL of the tested compounds. The WST-1 tetrazolium salt [sodium 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium] is reduced by mitochondrial dehydrogenases of viable cells to water-soluble formazan. The amount of formazan produced by viable cells was quantified by measuring the absorbance ($\lambda = 450$ nm). The anticancer activity of triterpenes were expressed as an IC₅₀ in μ M (Table 2).

Lipophilicity studies

The theoretical lipophilicity parameters of triazoles **5a-i** and **6a-j** were calculated using the commercially available ALOGPS 2.1 software program (Tetko et al. 2005) (Table 3).

Results and discussion

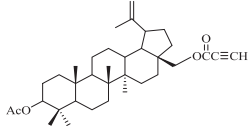
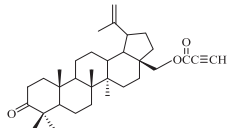
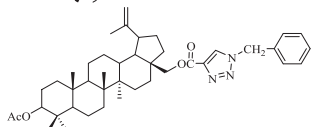
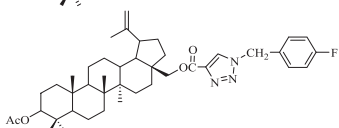
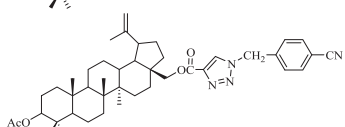
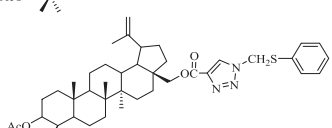
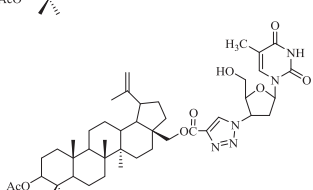
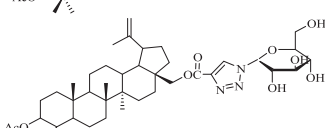
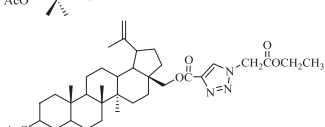
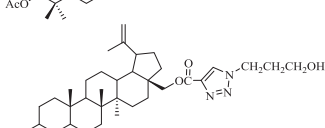
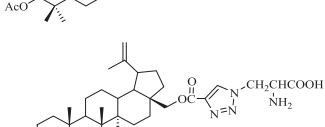
Chemistry

The synthesis of triazoles was started from betulin **1** and 3-acetylbetulin **2** (Fig. 1). Acetylation of betulin **1** at the C-3 and C-28 positions with acetic anhydride in the presence of 4-dimethylaminopyridine in pyridine gave betulin 3,28-diacetate. A selective hydrolysis of betulin 3,28-diacetate at C-28 position (MeOH/NaOH/THF) afforded 3-acetylobetulin **2** with a quantitative yield (Thibeault et al. 2007; Santos et al. 2010).

Subsequently, triterpenes **1–2** were used to prepare the propynoylated derivatives **3–4** according to our published procedures (Boryczka et al. 2013). The triazoles **5a-i** and **6a-j** were obtained by CuAAC reactions of acetylenic esters with various organic azides in toluene with yields in the range of 45–87%. Synthesis of triazoles **5a-i** and **6a-j** was depicted in Scheme 1. New compounds were purified by column chromatography on silica gel in CHCl₃–EtOH or CH₂Cl₂–EtOH with various ratios. The chemical characterization of all derivatives was carried out by ¹H-, ¹³C-NMR, IR spectroscopies, and HRMS spectra.

In the ¹H NMR spectra of the triazoles **5a-d** and **6a-d**, singlets of methylene group were observed at δ 5.48–5.69, which suggests the presence of a bond between C-4 (aryl group) and N-1 of the triazole ring. The signals in the range of δ 7.01–7.72 were assigned to the aromatic protons of the aryl group of derivatives **5a-d** and **6a-d**. Additionally, for all derivatives **5a-i** and **6a-j**, signals at δ 7.96–9.08 were

Table 2 Anticancer activity (IC_{50}) of acetylenic esters **3–4**, triazoles of triterpenes **5a–i** and **6a–j** and cisplatin as a reference compound against the tested human cancer cell lines

Compound	Chemical structure	Human Cell Line / $IC_{50} \pm SD$ [μM]		
		C-32	T47D	SNB-19
3		0.39 ± 0.05	Neg	0.67 ± 0.09
4		0.01 ± 0.001	50.75 ± 0.73	0.19 ± 0.06
5a		2.78 ± 0.61	Neg	49.91 ± 0.61
5b		0.74 ± 0.09	Neg	0.85 ± 0.07
5c		Neg	Neg	Neg
5d		6.64 ± 0.55	Neg	66.04 ± 1.53
5e		90.63 ± 5.73	62.56 ± 1.00	58.85 ± 0.48
5f		59.52 ± 0.58	44.25 ± 1.17	52.93 ± 1.01
5g		0.57 ± 0.08	Neg	0.78 ± 0.09
5h		18.86 ± 1.30	24.36 ± 1.37	7.75 ± 0.47
5i		21.84 ± 1.42	45.94 ± 2.10	1.22 ± 0.60

6a		Neg	Neg	Neg
6b		8.41 ± 0.11	Neg	0.45 ± 0.02
6c		6.42 ± 0.56	Neg	6.45 ± 0.13
6d		59.11 ± 0.83	83.88 ± 2.17	Neg
6e		0.81 ± 0.03	9.03 ± 0.57	0.17 ± 0.04
6f		0.76 ± 0.04	32.86 ± 1.28	7.45 ± 0.29
6g		Neg	Neg	Neg
6h		37.67 ± 2.02	14.63 ± 1.93	21.45 ± 1.56
6i		4.10 ± 0.19	7.56 ± 0.96	63.64 ± 0.79
6j		1.33 ± 0.71	23.91 ± 1.54	50.08 ± 1.92
Cisplatin		0.57 ± 0.06	38.40 ± 5.16	0.78 ± 0.08

Neg – negative in the concentration used

observed, corresponding to triazolyl protons in the 1,4-disubstituted triazole ring.

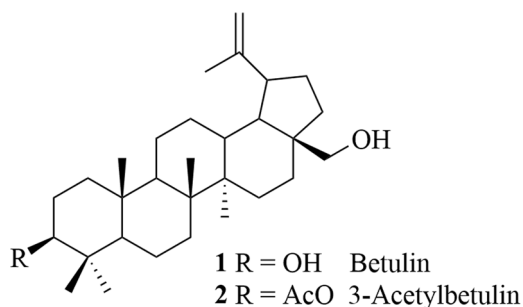
Analysis of the ^{13}C NMR spectra of triazoles **5a-i** and **6a-j** showed that the signals of acetyl and carbonyl groups are located at 167.5–171.1 p.p.m. and 217.0–218.1 p.p.m., respectively.

The IR spectra of new triazoles **5a-i** and **6a-j** showed characteristic absorption bands at 1527–1616 cm^{-1} and 1456–1458 cm^{-1} , which were attributed to the C=N and the N=N stretching vibrations of the triazole ring, respectively.

The HRMS negative mode was applied to identify all new compounds. In the mass spectra of triterpenes **3**, **5a-i**,

Table 3 The values of calculated lipophilicity parameters of compound **5a–i** and **6a–j**

Compound	ALOGPs	AC logP	ALOGP	MLOGP	XLOGP2	XLOGP3
5a	7.76	9.09	9.56	7.29	11.43	11.87
5b	6.97	7.00	9.13	7.63	10.63	10.95
5c	6.91	7.27	8.81	6.78	10.33	10.50
5d	7.19	9.60	9.52	7.44	11.03	11.43
5e	5.54	4.70	6.46	4.98	7.45	8.30
5f	4.54	3.99	5.19	3.99	6.85	6.96
5g	6.26	5.78	7.61	6.15	9.03	9.58
5h	5.85	5.75	6.87	5.98	8.55	8.80
5i	2.66	3.80	6.22	3.03	5.44	5.53
6a	6.62	7.13	8.51	6.78	9.26	9.89
6b	6.50	7.19	8.72	7.14	9.43	9.99
6c	6.58	6.94	8.39	6.39	8.99	9.61
6d	6.78	9.28	9.10	7.05	9.69	10.54
6e	5.04	4.38	6.04	4.56	6.11	7.41
6f	4.16	3.67	4.77	3.56	5.50	6.07
6g	5.90	5.45	7.20	5.72	7.68	8.69
6h	5.54	5.43	6.45	5.57	7.20	7.91
6i	2.37	3.47	5.80	2.59	4.10	4.64
6j	6.21	6.00	7.82	5.90	8.12	9.36

**Fig. 1** Chemical structure of betulin **1** and 3-acetylbetulin **2**

and **6a–j** signals based on ions $[M-H]^-$ were observed. These signals were corresponding to the calculated values.

Biological study

The triazole derivatives of 3-acetylbetulin and betulone were evaluated in vitro for their anticancer activity using a WST-1 assay against three human cancer cell lines: amelanotic melanoma C-32, ductal carcinoma T47D and glioblastoma SNB-19. Cisplatin was used as a positive control. The results of the anticancer activity tests of the studied compounds are reported in Table 2 as IC_{50} (μM).

As shown in Table 2, the lowest anticancer activity (IC_{50} 7.56–83.88 μM) of targeted triazoles was observed in the case of the T47D ductal carcinoma cell line. In the tested

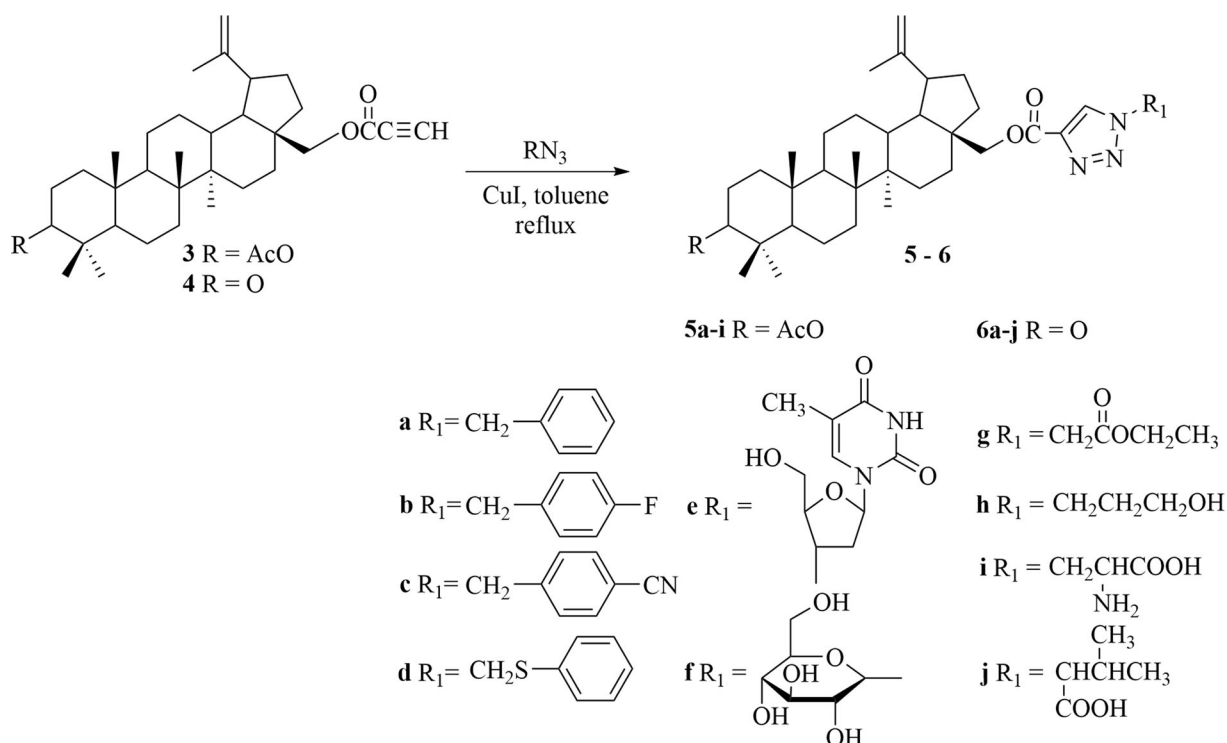
group of triazoles, derivative **6i** exhibited a highest anticancer activity (IC_{50} 7.56 μM) against the T47D cells, when compared to the positive control.

For triazoles of 3-acetylbetulin **5a–i**, the rank order of the anticancer activity towards the C-32 cell line is as follows: **5g** > **5b** > **5a** > **5d** > **5h** > **5i** > **5f** > **5e** > **5c**. The compound **5g** containing a 1-ethylacetyl moiety in triazole ring had the same anticancer activity against the C-32 cell line as the reference cisplatin (IC_{50} 0.57 μM). Moreover, triazoles **5c**, **6a**, and **6g** had no anticancer activity towards C-32 cell line in the applied concentration range.

According to our studies, compounds **5b**, **5g**, **6b**, and **6e** showed a significant activity against human glioblastoma SNB-19 cell line, with IC_{50} values from 0.17 to 0.85 μM .

The triazole **6e** bearing a 3'-deoxythymidine-5'-yl moiety showed the highest activity in the tested group of compounds against SNB-19 cells, with IC_{50} value of 0.17 μM .

Our studies suggest, that the introduction of acetyl or carbonyl group at the C-3 position of triazole derivatives of triterpenes afforded compounds having a higher anticancer activity against amelanotic melanoma C-32 cell line. Additionally, the compounds **5f** and **6f** containing the 1-deoxy- β -D-glucopyranosyl substituted triazole ring had a better activity than their parent 3-hydroxyl substituted analogs against C-32, T47D, and SNB-19 cell lines (Bębenek et al. 2017).



Scheme 1 Synthesis of triazole derivatives **5a-i** and **6a-j**. Reagents and conditions: organic azide (RN₃), CuI, reflux, 72 h

The lipophilicity is one of the important physicochemical parameters in drug development (Andrić and Héberger 2015). A lipophilicity study of the tested triazoles was carried out using the ALOGPS 2.1 software program. The predicted log P values were calculated according to the molecular structures of triazoles **5a-i** and **6a-j** using six computational methods (ALOGPs, AC logP, ALOGP, MLOGP, XLOGP2, and XLOGP3). Considering two triazoles of betulonone **6d** and **6e**, it was observed that their cytotoxic activity increased with the decreasing value of theoretical log P.

Conclusion

In conclusion, on the basis of the CuAAC reaction, a series of new derivatives of 3-acetylbetulin and betulonone bearing 1,2,3-triazole moiety has been synthesized. The anticancer activity of the triazoles and cisplatin was tested against the C-32, T47D and SNB-19 cancer cell lines using the WST-1 assay. The triazole **6e** with 3'-deoxythymidine-5'-yl substituent proved to be a potent cytotoxic agent with IC₅₀ value of 0.17 μM in the case of the human glioblastoma SNB-19 cell line. Moreover, the triazole **6e** can be considered as a promising candidate for anticancer therapy.

Acknowledgements This work was supported by the Medical University of Silesia in Katowice, Poland. Grant No KNW-1-015/K/7/0.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits use, duplication, adaptation, distribution, and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

- Andrić F, Héberger K (2015) Towards better understanding of lipophilicity: assessment of *in silico* and chromatographic log P measures for pharmaceutically important compounds by non-parametric rankings. *J Pharm Biomed Anal* 115:183–191
- Bębenek E, Jastrzębska M, Kadela-Tomanek M, Chrobak E, Orzechowska B, Zwolińska K, Latocha M, Mertas A, Czuba Z, Boryczka S (2017) Novel triazole hybrids of betulin: synthesis and biological activity profile. *Molecules* 22:1876–1892
- Bębenek E, Kadela-Tomanek M, Chrobak E, Wietrzyk J, Sadowska J, Boryczka (2016) New acetylenic derivatives of betulin and betulonone, synthesis and cytotoxic activity. *Med Chem Res* 26:1–8
- Bonaccorso HG, Moraes MC, Wiethan CW, Luz FM, Meyer AR, Zanatta N, Martins MAP (2013) Synthesis of 1*H*-1,2,3-triazoles-*Rufinamide* analogs by 1,3-dipolar cycloaddition and electrocyclization reactions of trifluoroacetyl enoethers under thermal solventless conditions. *J Flu Chem* 156:112–119

- Boryczka S, Bębenek E, Wietrzyk J, Kempieńska K, Jastrzębska M, Kusz J, Nowak M (2013) Synthesis, structure and cytotoxic activity of new acetylenic derivatives of betulin. *Molecules* 18:4526–4543
- Bräse S, Friedrich A, Gartner M M, Schröder T (2008) Cycloaddition reactions of azides including bioconjugation. *Top Heterocycl Chem* 12:45–115
- Dheer D, Singh V, Shankar R (2017) Medicinal attributes of 1,2,3-triazoles: current developments. *Bioorg Chem* 71:30–54
- He Y-W, Dong C-Z, Zhao J-Y, Ma L-L, Li Y-H, Aisa HA (2014) 1,2,3-Triazole-containing derivatives of rupestonic acid: click-chemical synthesis and antiviral activities against influenza viruses. *Eur J Med Chem* 76:245–255
- Khan I, Guru SK, Rath SK, Chinthakindi PK, Singh B, Koul S, Bhushan S, Sangwan PL (2016) A novel triazole derivative of betulinic acid induces extrinsic and intrinsic apoptosis in human leukemia HL-60 cells. *Eur J Med Chem* 108:104–116
- Majeed R, Sangwan PL, Chinthakindi PK, Khan I, Dangroo NA, Thota N, Hamid A, Sharma PR, Saxena AK, Koul S (2013) Synthesis of 3-O-propargylated betulinic acid and its 1,2,3-triazoles as potential apoptotic agents. *Eur J Med Chem* 63:782–792
- Marciniak K, Latocha M, Kurczab R, Boryczka S (2017) Synthesis and anticancer activity evaluation of a quinoline-based 1,2,3-triazoles. *Med Chem Res* 26:2432–2442
- Rostovtsev HV, Green LG, Fokin VV, Sharpless KB (2002) A step-wise Huisgen cycloaddition process: copper (I)-catalyzed regioselective “ligation” of azides and terminal alkynes. *Angew Chem Int Ed Engl* 41:2596–2599
- Santos RC, Salvador JAR, Marín S, Cascade M, Moreira JN, Dini TCP (2010) Synthesis and structure–activity relationship study of novel cytotoxic carbamate and N-acylheterocyclic bearing derivatives of betulin and betulinic acid. *Bioorganic Med Chem* 18:4385–4396
- Spivak AY, Gubaidullin RR, Galimshina ZR, Nedopekina DA, Odinokov VN (2016) Effective synthesis of novel C(2)-propargyl derivatives of betulinic and ursolic acids and their conjugation with β -D-glucopyranoside azides via click chemistry. *Tetrahedron* 71:1249–1256
- Suman P, Patel A, Solano L, Jampana G, Gardner ZS, Holt CM, Jonnalagadda SC (2016) Synthesis and cytotoxicity of Baylis-Hillman template derived betulinic acid-triazole conjugates. *Tetrahedron* 73:4214–4226
- Tetko IV, Gasteiger J, Todeschini R, Mauri A, Livingstone D, Ertl P, Palyulin VA, Radchenko EV, Zefirov NS, Makarenko AS, Tanchuk VY, Prokopenko VV (2005) Virtual computational chemistry laboratory-design and description. *J Comput Aid Mol Des* 19:453–463
- Thibeault D, Gauthier C, Legault J, Bouchard J, Dufour P, Pichette A (2007) Synthesis and structure-activity relationship study of cytotoxic germanicane- and lupane-type 3β -O-monodesmosidic saponins starting from betulin. *Bioorg Med Chem* 15:6144–6157
- Torne CW, Christensen C, Meldal M (2002) Peptidotriazoles on solid phase: [1,2,3]-triazoles by regioselective copper(I)-catalyzed 1,3-dipolar cycloadditions of terminal alkynes to azides. *J Org Chem* 67:3057–3064
- Totobenazara J, Anthony J, Burke AJ (2015) New click-chemistry methods for 1,2,3-triazoles synthesis: recent advances and applications. *Tetrahedron Lett* 56:2853–2859
- Wang X-L, Wan K, Zhou C-H (2010) Synthesis of novel sulfanilamide-derived 1,2,3-triazoles and their evaluation for antibacterial and antifungal activities. *Eur J Med Chem* 45:4631–4639
- Wei J, Chen J, Xu J, Cao L, Deng H, Sheng W, Zhang H, Cao W (2012) Scope and regioselectivity of the 1,3-dipolar cycloaddition of azides with methyl 2-perfluoroalkynoates for an easy, metal-free route to perfluoroalkylated 1,2,3-triazoles. *J Flu Chem* 133:146–154
- Yu F, Wang Q, Zhang Z, Peng Y, Qiu Y, Shi Y, Zheng Y, Xiao S, Wang H, Huang X, Zhu L, Chen K, Zhao C, Zhang C, Yu M, Sun D, Zhang L, Zhou D (2013) Development of oleanane-type triterpenes as a new class of HCV entry inhibitors. *J Med Chem* 56:4300–4319